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Telomere length is an independent prognostic marker in MDS but not in de novo AML

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Telomere length is prognostic in MDS but not AML

Telomere length is an independent prognostic marker in MDS but not in de novo AML

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Telomere length is prognostic in MDS but not AML

30 Summary

31 Telomere dysfunction is implicated in the generation of large-scale genomic rearrangements
32 which drives progression to malignancy. In this study we used high-resolution single
33 telomere length analysis (STELA) to examine the potential role of telomere dysfunction in 80
34 Myelodysplasia (MDS) and 95 de novo Acute Myeloid Leukaemia (AML) patients. Despite
35 the MDS cohort being older they had significantly longer telomeres than the AML cohort (P
36 $<.0001$) where telomere length was also significantly shorter in younger AML patients (age
37 <60) ($P = .02$) and in FLT3 ITD mutated AML patients ($P = .03$). Using a previously determined
38 telomere length threshold for telomere dysfunction (3.81kb) did not provide prognostic
39 resolution in AML ($HR = 0.68$, $P = .2$). In contrast, the same length threshold was highly
40 prognostic for overall survival in the MDS cohort ($HR = 5.0$, $P <.0001$). Furthermore, this
41 telomere length threshold was an independent parameter in multivariate analysis when
42 adjusted for age, gender, cytogenetic risk group, number of cytopenias and IPSS score ($HR =$
43 2.27 , $P <.0001$). Therefore, telomere length should be assessed in a larger prospective study
44 to confirm its prognostic role in MDS with a view to integrating this variable into a revised
45 IPSS.

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46 **Introduction**

47 The Myelodysplastic Syndromes (MDS) are a heterogeneous group of clonal haematopoietic
48 disorders with varying survival and propensity to develop secondary acute myeloid
49 leukaemia (sAML). In 1997 the International Prognostic Scoring System (IPSS) was based on
50 the bone marrow blast count, 3 distinct cytogenetics risk groups and the number of
51 cytopenias dividing patients into four IPSS subgroups(Greenberg, *et al* 1997). The IPSS – R
52 (Revised) is an updated refinement of the IPSS which identifies five cytogenetic, three
53 cytopenic and four blast count risk categories which combine into five overall prognostic
54 subgroups(Greenberg, *et al* 2012, Vardiman, *et al* 2009). Many MDS patients have a normal
55 karyotype but in recent years a very large number (up to 660) of molecular genetic defects
56 have been identified encoding genes for cellular proteins including transcription factors e.g.
57 *RUNX1*, epigenetic regulators and chromatin remodelling factors e.g. *TET2*, *DNMT3A*,
58 *IDH1/2*, pre-RNA splicing factors e.g. *SF3B1*, receptor tyrosine kinase/ signalling molecules
59 e.g. *NRAS*, *JAK2*, *NPM1*,*FLT3* and check point regulator *P53* some of which impact
60 significantly on prognosis (Tothova, *et al* 2013, Walter, *et al* 2012). A recent MDS study
61 showed that some of these somatic mutations were an independent prognostic marker
62 compared to the IPSS (Bejar, *et al* 2011).

63 Recent studies have shown that the same genomic mutations seen in MDS/AML patients
64 overlap with those identifiable in the normal adult population with increasing frequency
65 with age (Genovese, *et al* 2014, Jaiswal, *et al* 2014, McKerrell, *et al* 2015, Xie, *et al* 2014).
66 What drives these clones to further genomic instability and the development of MDS/AML is
67 currently poorly understood. Telomeres are repetitive DNA sequences at the ends of
68 chromosomes that shorten with each cell division. Critical loss of telomere length leads to
69 chromosome end-end fusion and genomic instability resulting in large scale re-

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arrangements such as non-reciprocal translocations which are the hallmark of many tumour types including MDS, sAML and *de novo* AML (Jones, *et al* 2012). With ongoing cell division during life, the telomeres in normal individuals erode as a function of age at a rate of approximately 26 bp/year (Daniali, *et al* 2013). Several studies have suggested that MDS is associated with shorter telomeres leading to genomic instability and progression to sAML (Boulton, *et al* 1997, Chakraborty, *et al* 2009, Sieglöva, *et al* 2004, Townsley, *et al* 2014, Young 2010). Recently, using a modified Q-FISH based method, Gadji *et al* proposed that telomere dysfunction underpins the chromosomal changes associated with MDS progression to AML and *de novo* AML (Gadji, *et al* 2012). Whilst in a mouse model it was shown that telomere dysfunction induced the same types of DNA damage that drives classical MDS phenotypes including *SF3B1* and *DNMT3A* resulting in differentiation changes in myeloid precursors (Colla, *et al* 2015).

We previously developed a high-resolution technique to determine telomere length, Single Telomere Length Analysis (STELA), which is unique in its ability to detect telomere lengths from single chromosomes within the length ranges at which telomere fusions can occur (Lin, *et al* 2010, Lin, *et al* 2014). Using STELA we have shown that some chronic lymphocytic leukaemia (CLL) patients display extreme telomere shortening and fusion consistent with the onset of a telomere-driven crisis that can drive the formation of large-scale genome rearrangements (Lin, *et al* 2010). Telomeres in these ranges cannot be readily detected with the other methodologies such as QPCR, Southern blot or Q-FISH previously used in MDS/AML studies (Baird, *et al* 2003, Britt-Compton, *et al* 2012, Chakraborty, *et al* 2009, Lin, *et al* 2010). We showed that in CLL patients, telomere fusions only occurred when telomere length was ≤ 3.81 kb and importantly we show that telomere erosion to within these length

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93 ranges precedes clinical progression. Indeed high-resolution telomere length analysis using
94 STELA, together with the stratification of patients based on the telomere length thresholds
95 at which fusion occurs, provided an independent high-resolution marker of prognosis even
96 in patients with early-stage CLL (Lin, *et al* 2014).

97 In this study we used STELA to assess if telomere erosion is an important pathogenic
98 mechanism driving prognosis MDS and AML.

99 **Methods**

100 **Patients, samples and cell separation**

101 This study was undertaken at the University Hospital of Wales (UHW), Cardiff. All of the
102 unselected diagnostic patient bone marrow samples: 80 MDS which consisted of 37 samples
103 from Dundee (archived from 1997 – 2005) and 43 from Cardiff (archived between 1985 and
104 2008) including 7 with a proven and documented history of MDS prior to progression to
105 AML (blasts >20%), and 95 de novo AML from Cardiff (archived between 2003 and 2012)
106 were obtained following written informed consent (Table 1). We deliberately chose to use
107 stored but well annotated archival samples so as to be able to assess the potential impact of
108 telomere length on survival in all patient groups but especially low risk MDS patients. All
109 MDS patients were treated with best supportive care with none receiving azacitidine prior
110 to this analysis. All morphological, immunophenotypic, cytogenetic and molecular data
111 were collected from Cardiff and Dundee Haematology Departments for their respective
112 patients but all telomere length analyses on the bone marrow mononuclear cells were
113 undertaken within the Division of Cancer and Genetics, Cardiff University. **Bone marrow**
114 **mononuclear cells were collected in ethylenediaminetetraacetic acid and isolated by**
115 **density centrifugation using Ficoll-Hypaque (Invitrogen) which resulted in <3%**

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lymphocytes contamination. Cells were stored at -20°C as dry pellets before DNA extraction.

DNA extraction, single telomere length analysis and telomerase assays.

Telomere length was determined using XpYp STELA as previously described (Lin, *et al* 2010, Roger, *et al* 2013). Briefly, DNA was extracted using proteinase K, RNase A, phenol/chloroform protocols and quantified by Hoechst 33258 fluorometry (Bio-Rad) before dilution to $10\text{ ng}/\mu\text{L}$ in 10mM Tris-HCl, pH 7.5. A total of 10 ng of DNA was further diluted to $250\text{ pg}/\mu\text{L}$ in a volume of $40\text{ }\mu\text{L}$ containing $1\mu\text{M}$ Telorette2 linker and 1mM Tris-HCl, pH 7.5. Multiple polymerase chain reactions (PCRs; typically 6 reactions per sample) were carried out for each test DNA in $10\text{-}\mu\text{L}$ volumes 250 pg of DNA, $0.5\mu\text{M}$ of the telomere-adjacent and Teltail primers, 75mM Tris-HCl, pH 8.8, 20mM $(\text{NH}_4)_2\text{SO}_4$, 0.01% Tween-20, 1.5mM MgCl_2 , and 0.5 U of a $10:1$ mixture of Taq (ABGene) and Pwo polymerase (Roche Molecular Biochemicals). The reactions were cycled with an MJ PTC-225 thermocycler (MJ Research). The DNA fragments were resolved by 0.5% Tris acetate ethylenediaminetetraacetic acid agarose gel electrophoresis, and detected by Southern blot hybridization with random-primed $\alpha\text{-}^{33}\text{P}$ -labelled (GE Healthcare) TTAGGG repeat probe together with probes to detect the 1-kb (Stratagene) and 2.5-kb (Bio-Rad) molecular weight markers. The hybridized fragments were detected by phosphorimaging with a Molecular Dynamics Storm 860 phosphorimager (GE Healthcare). The molecular weights of the DNA fragments were calculated using the Phoretix 1D quantifier (Nonlinear Dynamics). Telomerase assays were undertaken using the TRAPeze XL Telomerase detection kit (Chemicon International, Billerica, MA) as previously described (Lin, *et al* 2010, Roger, *et al* 2013).

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139 **Statistical methods**

140 Statistical analysis was undertaken by the Haematology Clinical Trials Unit, Cardiff University
141 using SAS version 9.4 and GraphPad Prism 6. Spearman’s correlation was used for
142 correlations between baseline values; Mann-Whitney and Wilcoxon matched-paired
143 nonparametric tests were used for comparisons between groups. Mean telomere length
144 was assessed between diagnoses using the Wilcoxon rank sum test. Paired data were
145 compared using the Wilcoxon signed rank test. **In the MDS cohort patients were assessed**
146 **using the 1997 IPSS criteria for the 3 cytogenetic risk groups, blast count, number of**
147 **cytopenias (Hb <100g/l, neutrophils <1.5x10⁹/l and platelets <100x10⁹/l), IPSS score, age**
148 **and sex whereas the AML cohort was assessed for cytogenetic risk group and FLT3 and**
149 **NPM mutations (Falini, *et al* 2005, Kottaridis, *et al* 2001, Townsley, *et al* 2014).** Survival
150 was assessed using the Kaplan-Meier method, and compared using Cox proportional
151 hazards regression, with model building carried out using forward selection with significance
152 set at P = .05.

153 **Results**

154 **Telomere length MDS and AML patients.**

155 An MDS patient’s bone marrow will typically display a variable range of immature and more
156 differentiated cells which may or may not be derived from the malignant stem cells. We
157 therefore compared the telomere length of the first 20 individual MDS patients CD34+ and
158 CD34- bone marrow cells, but found no significant difference in telomere length between
159 the two fractions (P = .08; Supplementary Figure 1A). We therefore proceeded without cell

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selection in subsequent MDS patient bone marrow sample analyses and simply used bone marrow mononuclear cell pellets.

Despite the fact that the MDS patients (median age 68 years, range 21-86) were older than AML patients (median age 56 years, range 17-80) telomere length was significantly longer in the MDS cohort compared to the AML cohort ($P < .0001$; Figure 1A). Although MDS samples showed a modest reduction in telomere length with increasing age at diagnosis, there was no significant correlation ($\rho^2 = -.0212$, $P = .2$; Figure 1B) whereas in the AML samples a positive correlation was observed ($\rho^2 = .0890$, $P = .003$; Figure 1C). This finding is in contrast to what occurs during normal ageing; samples derived from older AML patients (age >60) had significantly longer telomeres than younger AML patients ($P = .02$; Figure 1D). Indeed, 22/26 (84.6%) of AML patients age <50 years had telomere lengths within the range at which they can become dysfunctional or 'fusogenic' (≤ 3.81 kb), which we previously described in CLL (Lin, *et al* 2014).

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174 **Telomere length, blast count, cytogenetics, cytopenias and IPSS.**

We next analysed the MDS cohort to assess any possible correlations between telomere length and age, gender, blast count, number of cytopenias, cytogenetic risk group and IPSS sub-group. Shorter telomere length was associated with male gender ($P = .01$; Supplementary Figure 1B) and increased number of cytopenias ($P = .003$; Figure 2A). None of the other parameters were significantly associated with telomere length. Consistent with previous reports we found a significant association between the number of cytopenias and overall survival ($P < .0001$; Supplementary Figure 2A). Furthermore, patients with high risk

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cytogenetic abnormalities tended to have worse survival although this did not reach statistical significance ($P = .12$; Supplementary Figure 2B). Interestingly we found no simple association between telomere length and the three IPSS cytogenetic risk groups ($P = .6$ for trend; Figure 2B), the four IPSS subgroups ($\rho^2 = .14$ for correlation; Figure 2C) or bone marrow blast percentage ($\rho^2 = -0.22$, $P = .0503$ Figure 2D).

In the de novo AML cohort we assessed the correlation between telomere length and gender, age, presenting WBC, performance status, whether primary or secondary AML and NPM1 and FLT3 mutation status. There was no association between NPM1 mutated patients and telomere length (data not shown), but significantly shorter telomeres were found in the FLT3 ITD mutated group when compared to FLT3 wild type AML patients ($P = .03$). In contrast, there was a trend towards longer telomeres in the FLT3 TKD mutated group compared to ITD mutated AML ($P = .12$; Figure 2E).

Telomerase activity in MDS and AML patients.

Given the different telomere length characteristics of the MDS and AML cohorts, we investigated whether this may reflect differences in telomerase activity, an enzyme responsible for extending shortened telomeres. Telomerase activity was analysed in a subset of CD34+ AML samples ($n = 12$) and in purified CD34+ cells from MDS patients ($n = 20$). Telomerase activity was significantly higher in the AML samples when compared with CD34+ MDS cells ($P = .0002$; Figure 2F).

Telomere length and survival in MDS and AML.

Telomere length is prognostic in MDS but not AML

204 We next assessed the impact of telomere length on overall survival in the MDS and AML
205 cohorts. Segregation of the two cohorts according to whether their mean telomere length
206 was above or below the upper limit of telomere dysfunction (3.81 kb) revealed no
207 difference in survival in the AML patient group (HR = 0.68 (0.37-1.9), $P = .2$; Figure 3A). In
208 contrast, bifurcation of the MDS cohort using this threshold telomere length demonstrated
209 that patients with a median telomere length ≤ 3.81 kb had significantly worse survival (HR =
210 5.0 (2.7-10.0), $P < .0001$; Figure 3B). The impact of telomere length in these two disease
211 settings was confirmed using telomere length quartile analysis. There was no correlation
212 between telomere length and overall survival or relapse-free survival in the AML cohort ($P =$
213 $.5$, $P = .09$; Supplementary Figures 3A and 3B). However, similar quartile analysis in the MDS
214 cohort clearly showed that patients in the lowest two quartiles had worse survival with all
215 patients in the lowest quartile alive at 3 years (Supplementary Figure 3C).

217 **Telomere length is an independent prognostic variable in MDS.**

218 Finally, we performed multivariate analysis in the MDS cohort using a forward selection
219 model that included age, IPSS, gender, cytogenetic risk group, number of cytopenias and
220 telomere length. Short telomere length was identified as the most significant independent
221 marker of overall survival (HR = 2.27 (1.45-3.57), $P < .0001$). When entering short telomere
222 length into the model the following parameters retained independent prognostic value: high
223 IPSS (HR = 1.2 (0.64-2.27), increased number of cytopenias (HR = 1.60 (1.09-2.35), $P = .007$),
224 older age (HR per year 1.03 (1.01-1.07), $P = .05$) and male gender (HR = 2.70 (1.20-6.10), $P =$
225 $.01$).

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229 **Discussion**

230 We previously showed that a proportion of chronic lymphocytic leukaemia (CLL)
231 patients display extreme telomere erosion and fusion consistent with a telomere-driven
232 crisis. Importantly, this was not simply a function of advanced stage disease but was
233 detected in a subset of early stage patients prior to clinical progression (Lin, *et al* 2010).
234 Subsequently we showed that defining the specific telomere length threshold at which
235 telomere fusion occurred was a powerful way to risk-stratify CLL patients even those with
236 early-stage disease (Lin, *et al* 2014). Previous studies had suggested a possible role for
237 telomere dysfunction in both MDS and AML, so here we investigated the relationship
238 between telomere length, disease progression and clinical outcome in MDS and AML using
239 the high-resolution STELA technique.

240 We demonstrated that telomere length was highly predictive of disease outcome in MDS. In
241 contrast, we found no evidence that telomere length influenced the survival of de novo AML
242 patients. It should be noted, however, that we demonstrated shorter telomere lengths in
243 the FLT3-ITD mutated group, a group with a well-established poorer prognosis. These results
244 are similar to those of Aalbers *et al* who also showed shorter telomeres in patients with
245 FLT3-ITD mutations but not NPM1 mutated patients (Aalbers, *et al* 2013). One possible
246 explanation for the different telomere length characteristics in MDS and AML may be the
247 differential expression of telomerase found in these two conditions. Unlike MDS cells, AML
248 cells showed evidence of upregulated telomerase activity, which could prevent replicative

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senescence and allow unlimited proliferation during leukaemogenesis (Engelhardt, *et al* 2004, Shay and Wright 2011). MDS is characterised by clonal expansion and hypercellularity but increased apoptosis leading to cytopenias (Greenberg 1999, Greenberg 1998, Parker, *et al* 1998, Parker and Mufti 2000). The reason why telomerase is not upregulated in MDS is unknown, but to date studies have failed to show any responsible acquired telomerase-regulated genetic abnormalities in this patient group, although there are several germline mutations described in TERC and TERT predisposing to MDS/AML (Ballew and Savage 2013, Calado, *et al* 2009, Kirwan, *et al* 2009, Ohyashiki, *et al* 1999, Yamaguchi, *et al* 2003).

Our study showed an association between telomere length and the number of cytopenias in MDS patients. It seems possible that the lack of telomerase activity observed in MDS leads to unhindered cell senescence and/or apoptosis. Also the high proliferative and apoptotic rates seen in MDS bone marrow produces increased ineffective haematopoiesis and more profound cytopenias (Raza, *et al* 1997a, Raza, *et al* 1997b). These results are similar to those of Sieglöva *et al* who showed MDS patients with shorter telomeres were more likely to progress to AML (Sieglöva, *et al* 2004). Perhaps surprisingly, there was no association between telomere length and cytogenetic risk group, blast count and IPSS score. This lack of association meant that telomere length was an independent marker of outcome in MDS and in the multivariate forward selection model we employed. Indeed, it was more prognostic than blast count, cytogenetics, number of cytopenias or IPSS in this context.

Several recent studies have shown that clonal haematopoiesis is almost a “normal” part of ageing with recent reports showing 0.8%, 11% and 19.5 % of normal individuals aged <60, >80 and >90 years respectively having demonstrable clonal haematopoiesis – so called age-related clonal haematopoiesis. These include the acquisition of many genetic lesions

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3 272 associated with the development of MDS/AML including DNMT3, IDH1, IDH2, NRAS, KRAS,
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5 273 JAK 2, SF3B1 and SRSF2 mutations (Jaiswal, *et al* 2014, McKerrell, *et al* 2015, Xie, *et al* 2014).
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7 274 Three of our observations in this study were that the telomere length increases with age in
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9 275 AML patients, that AML patients older than 60 years having significantly long telomeres
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11 276 than those age <60 years and that the AML patient cohort had significantly shorter
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13 277 telomeres than the MDS patient cohort despite being younger. One possible explanation for
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15 278 these data is that older de novo AML patients may have developed secondary AML despite
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17 279 the absence of a documented history of prior MDS or clonal haematopoiesis. This would be
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19 280 in keeping with the recent identification of increased genomic instability in ageing “normal”
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21 281 people with many identical genomic abnormalities seen in elderly AML patients (McKerrell,
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23 282 *et al* 2015). The acquisition of the various differing leukaemia-associated genomic
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25 283 abnormalities are age dependent with for example the recurrent point mutations affecting
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27 284 spliceosome genes SF3B1 and SRSF2 associated with clonal haematopoiesis in individuals
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29 285 aged 70 years or over, but not in younger people (McKerrell and Vassiliou 2015,
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31 286 Papaemmanuil, *et al* 2013). An alternative, but not exclusive hypothesis, is that AML in
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33 287 younger patients tends be more progenitor-type AML (Core binding factor, NPM1-mutated,
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35 288 FLT3-ITD-mutated) and more proliferative leading to shorter telomeres. Further studies are
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37 289 required to assess the relationship of shorter telomeres with age-related clonal
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39 290 haematopoiesis and MDS and AML development.
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49 291 The findings in MDS are in keeping with our previous data in CLL and breast cancer where
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51 292 we demonstrated the utility of our telomere-length threshold in providing powerful
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53 293 independent prognostic information (Lin, *et al* 2014, Roger, *et al* 2013). Taken together
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55 294 these data point to a common mechanism that is present in diverse tumour types, by which
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Telomere length is prognostic in MDS but not AML

295 the presence of short dysfunctional telomeres can drive genomic instability and clonal
296 evolution leading to poor clinical outcomes.

297 Our study does have several limitations in that it consists of relatively small cohorts of MDS
298 and AML patients and was deliberately retrospective so as to be able to assess the impact of
299 telomere length on survival especially in low risk MDS patients, all of whom were treated
300 with supportive care only. Finally our observation that telomere length is independently
301 prognostic in MDS indicates that consideration should be given to a much larger prospective
302 study assessing the potential role of telomeres in the prognostication of MDS. This would
303 also facilitate the assessment of telomere length analysis as a potential predictor of
304 response to newer therapies such as azacitidine and perhaps its ultimate incorporation into
305 a revised IPSS.

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310 Designed the research study – CF, CP, DMB

311 Contributed essential reagents or tools –ST, MG, DTB, SK

312 Analysed the data – CP, DMB, RKH

313 Wrote the paper – CP, DMB, CF

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Figures and Legends.

Table 1. Demographics of MDS and AML Cohorts

Figure 1. (A) Telomere length in the MDS cohort was significantly longer than those of the AML cohort despite being older median age 68 v 56 years ($p < .0001$). B) In MDS patients there was no correlation between telomere length and age of diagnosis ($\rho^2 = .0212$; $P = .2$). (C) In AML patients there was a positive correlation between telomere length and age of diagnosis ($\rho^2 = .0890$; $P = .003$). (D) Older AML patients (age >60) had significantly longer telomeres than younger patients ($P = .02$).

Figure 2. (A) In the MDS cohort there was a significant association between the telomere length and the number of cytopenia but not with (B) cytogenetic risk group ($P = .6$ for trend) or (C) IPSS sub-groups ($\rho^2 = .14$ for correlation) or (D) blast counts ($\rho^2 = .22$ $P = .503$). (E) Patients with a FLT3-ITD showed significantly shorter telomeres than the FLT3-WT group (overall $P = .03$ and there was a trend towards shorter telomere length in the FLT3-ITD group when compared with the FLT3-TKD group ($P = .12$)). F) Telomerase activity was significantly higher in AML CD34 + cells compared to MDS CD34+ cells ($P = .0002$).

Figure 3. (A) Using our previously described CLL fusogenic length threshold (TL ≤ 3.81 kb) there is no difference in survival in AML patients (HR = 1.47 (0.80-2.68), $P = 0.2$). (B) In contrast, categorization of the MDS cohort above and below the fusogenic length threshold (3.81 kb) demonstrated that MDS patients with short telomeres had an inferior survival (HR = 5.0 (2.7-10.0), $P < .0001$).

Supplementary Figure 1. (A) In 20 MDS patients bone marrow mononuclear cells were sorted but there was no significant difference in telomere length in CD34+ and CD34 – selected sub-populations. (B) In the MDS cohort longer telomere length was associated with female sex ($P = .01$).

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Supplementary Figure 2. (A) In the MDS cohort there was a significant association between the number of cytopenias and overall survival ($P < .0001$). (B) In the MDS cohort patients with high risk cytogenetic lesions tended to have worse survival although this did not reach statistical significance probably due to the relatively small sample size ($P = .12$).

Supplementary Figure 3. Quartile analysis of telomere length revealed no significant difference in (A) overall survival and (B) relapse-free survival in the de novo AML cohort. In contrast, (C) MDS patients in the lower two telomere length quartiles showed significantly shorter overall survival.

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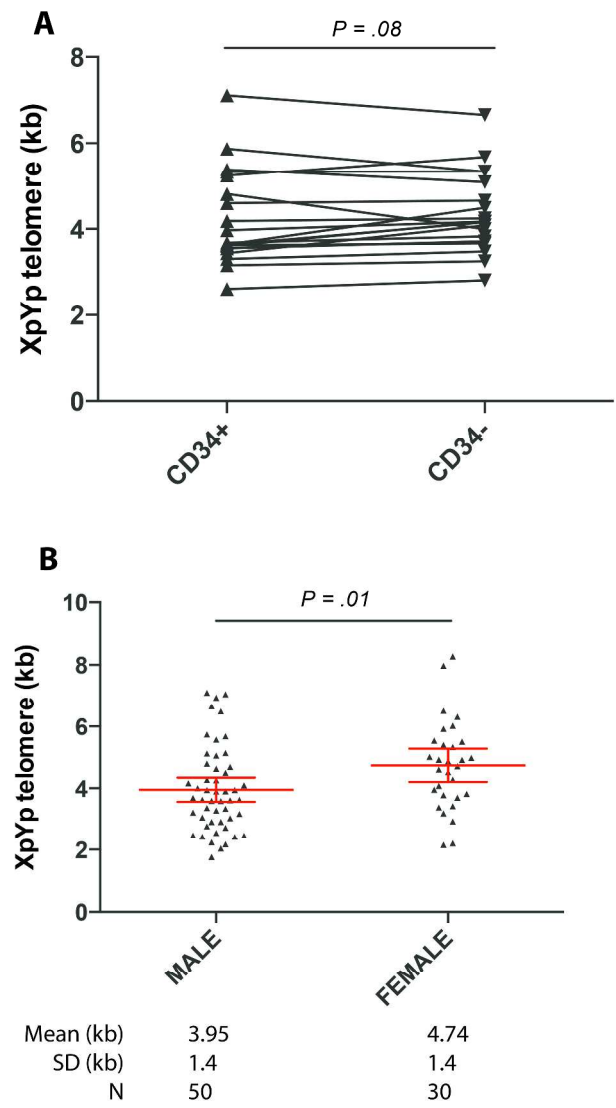
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Demographic Feature	MDS Cohort (n=80)	AML Cohort (n=95)
Sex		
Male	50	48
Female	30	47
Age		
Median (range)	68 (21-86)	56 (17-80)
<60	20	56
≥60	60	39
Cytogenetic Risk Group		
Good	45	6
Intermediate	15	76
Poor	15	3
Failed/Not known	5	10
Number of cytopenias		
1	37	
2	15	
3	28	
Bone Marrow Blast Count		
<5%	36	
5-10%	13	
10-20%	24	
>20%	7	
IPSS Score		
High	25	
Intermediate-2	11	
Intermediate-1	19	
Low	25	
Treatment		
Intensive		89
Non-intensive		6
ITD		
WT		49
Mutant		39
Unknown		7
NPM1c		
WT		35
Mutant		54
Unknown		6
TKD		
WT		73
Mutant		19
Unknown		3
WBC		
Median (range)		34.1 (1.3-294.0)
Performance Status (WHO)		
0		54
1		30
2		4
3+		7

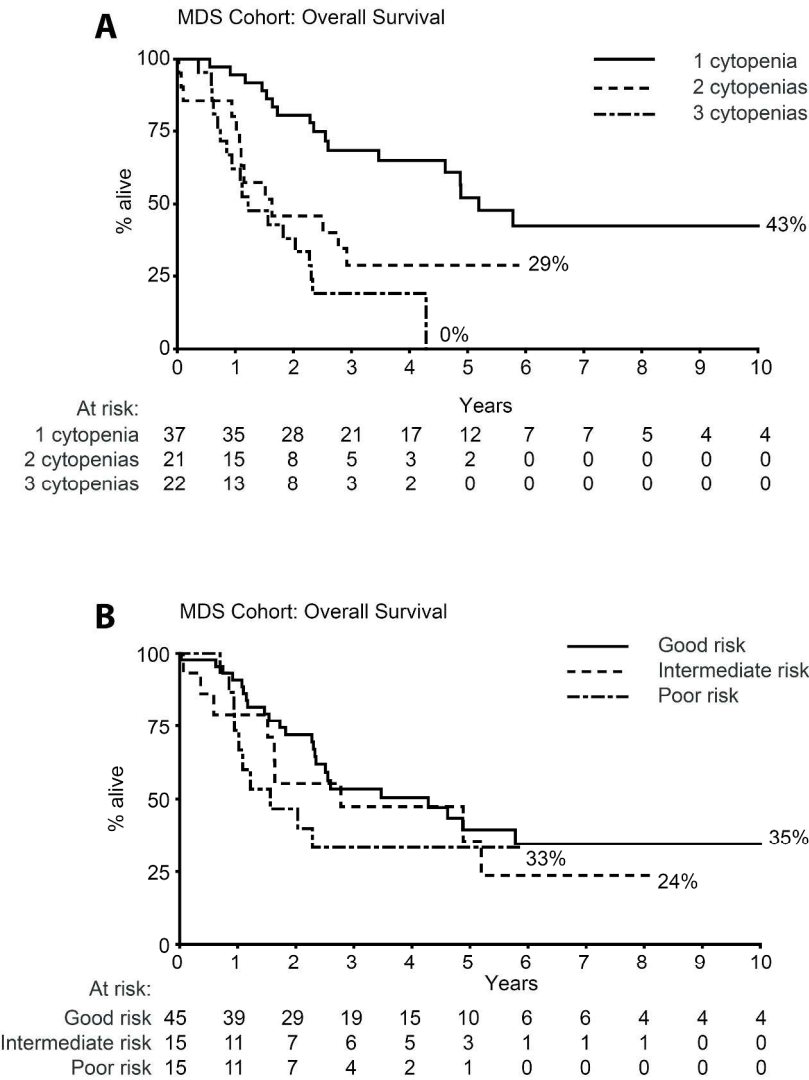
Williams et al_Supplementary Figure 1



Supplementary Figure 1

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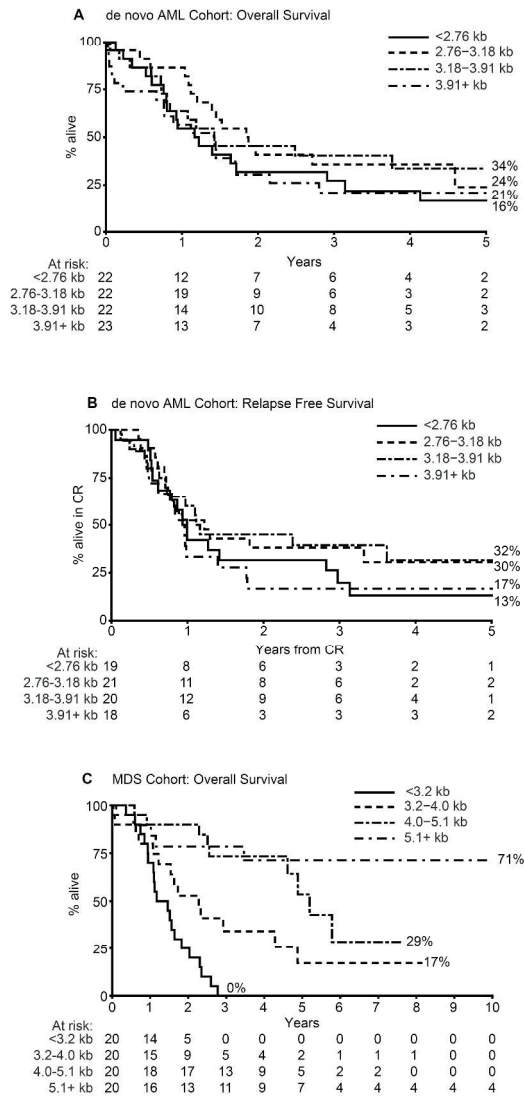
Williams et al_Supplementary Figure 2



Supplementary Figure 2

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Williams et al_Supplementary Figure 3



Supplementary Figure 3

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